

CLAIMS

We claim:

- 5 1. A transgenic nematode, the cells of which contain a transgene comprising a regulatory element of a gene that encodes a nematode secretory product or a homolog thereof operably linked to a DNA sequence encoding a detectable marker.
2. The transgenic nematode of claim 1, wherein the transgene further comprises at least a
10 portion of the coding sequence of the gene.
3. The transgenic nematode of claim 2, wherein the transgene further comprises an intron from the gene.
- 15 4. The transgenic nematode of claim 2, wherein the transgene further comprises at least a portion of the 3' untranslated region from the gene.
- 20 5. The transgenic nematode of claim 2, wherein the coding sequence of the gene is in frame with the sequence encoding the detectable marker.
6. The transgenic nematode of claim 1, wherein the transgene is contained in a chromosome.
7. The transgenic nematode of claim 1, wherein the transgene is extrachromosomal.
- 25 8. The transgenic nematode of claim 5, wherein the transgene comprises an integrated array comprising a second regulatory element operably linked to a second copy of a DNA sequence encoding the detectable marker.

9. The transgenic nematode of claim 8, wherein the second regulatory element directs expression of the detectable marker in a substantially different population of cells to that in which the first regulatory element directs expression of the detectable marker.

5 10. The transgenic nematode of claim 1, wherein the nematode secretory product is a protein.

11. The transgenic nematode of claim 1, wherein the detectable marker is selected from the list consisting of: a fluorescent polypeptide, a chemiluminescent polypeptide, an epitope tag, and an enzyme.

10 12. The transgenic nematode of claim 1, wherein the detectable marker is selected from the list consisting of: green fluorescent protein, luciferase, chloramphenicol acetyl transferase, xanthine-guanine phosphoribosyl transferase, beta-galactosidase, a Myc tag, and an HA tag.

15 13. The transgenic nematode of claim 1, wherein the detectable marker comprises a variant of a marker selected from the list consisting of: green fluorescent protein, luciferase, chloramphenicol acetyl transferase, xanthine-guanine phosphoribosyl transferase, beta-galactosidase, a Myc tag, and an HA tag, wherein the variant is detectable using the same detection means by which the marker of which it is a variant is detectable.

20 14. The transgenic nematode of claim 1, wherein the transgenic nematode is *C. elegans*.

25 15. The transgenic nematode of claim 1, wherein the transgenic nematode is *C. elegans* and wherein the regulatory element from a nematode secreted product or homolog thereof comprises a regulatory element from a gene that encodes a *C. elegans* homolog of a parasitic nematode secreted protein.

16. The transgenic nematode of claim 1 or claim 2, wherein the transgenic nematode is *C. elegans* and wherein the regulatory element from a nematode secreted product or homolog thereof comprises a regulatory element from a gene that encodes a *C. elegans* homolog of a parasitic nematode secreted protein, wherein the parasitic nematode secreted protein is found naturally in a nematode that is a member of an order selected from the group consisting of the *Strongylida*, *Rhabditida*, *Ascaridida*, *Spirurida*, *Oxyurida*, *Enoplida*, *Tylenchida*, or *Dorylaimida* nematode orders.

17. The transgenic nematode of claim 1 or claim 2, wherein the transgenic nematode is *C. elegans* and wherein the regulatory element from a nematode secreted product or homolog thereof comprises a regulatory element from a gene that encodes a *C. elegans* homolog of a parasitic nematode secreted protein, wherein the parasitic nematode secreted protein is found naturally in a nematode that is a member of a genus selected from the list consisting of the *Haemonchus*, *Oestertagia*, *Trichostrongylus*, *Cooperia*, *Dictyocaulus*, *Strongylus*, *Oesophagostomum*, *Syngamus*, *Nematodirus*, *Heligmosomoides*, *Nippostrongylus*, *Metastrongylus*, *Angiostrongylus*, *Ancylostoma*, *Necator*, *Uncinaria*, *Bunostomum*, *Strongyloides*, *Steinernema*, *Ascaris*, *Parascaris*, *Toxocara*, *Toxascaris*, *Baylisascaris*, *Anisakis*, *Pseudoterranova*, *Heterakis*, *Wuchereria*, *Brugia*, *Onchocerca*, *Dirofilaria*, *Loa*, *Thelazia*, *Dracunculus*, *Gnathostoma*, *Enterobius*, *Oxyuris*, *Syphacia*, *Trichinella*, *Trichuris*, *Capillaria*, *Globodera*, *Heterodera*, *Meloidogyne*, *Anguina*, *Ditylenchus*, *Hirschmanniella*, *Nacobus*, *Pratylenchus*, *Radopholus*, *Criconema*, *Tylenchulus*, *Paratylenchus*, *Aphelenchus*, *Bursaphelenchus*, *Longidorus*, *Xiphinema*, *Trichodorus*, and *Paratrichodorus* nematode genera.

18. The transgenic nematode of claim 15, wherein the *C. elegans* homolog of a parasitic nematode secreted product is the *C. elegans* ortholog of the parasitic nematode secreted product.

19. The transgenic nematode of claim 1, wherein the nematode secreted product comprises a molecule that is present in a *C. elegans* external secretion, wherein the *C. elegans* is a wild type or transgenic *C. elegans*.

20. The transgenic nematode of claim 1, wherein the nematode secreted product comprises an expression product of a gene that is expressed in a *C. elegans* secretory cell, tissue, or organ, wherein the gene comprises a nucleotide sequence that encodes a signal peptide.

21. The transgenic nematode of claim 1, wherein the nematode secretory product comprises a *C. elegans* homolog of a parasitic nematode secretory product, wherein the parasitic nematode secretory product is present in external secretions of the parasitic nematode.

22. The transgenic nematode of claim 1, wherein the nematode secretory product comprises a *C. elegans* homolog of a putative parasitic nematode secretory product, wherein the putative parasitic nematode protein contains a signal sequence.

23. The transgenic nematode claim 1, wherein the nematode secretory product comprises a *C. elegans* homolog of a putative parasitic nematode secretory product, wherein the putative parasitic nematode secretory product is present in a specialized secretory cell.

24. The transgenic nematode of claim 1, wherein the regulatory element is a *C. elegans* regulatory element.

25. The transgenic nematode of claim 1, wherein the regulatory element is a 5' regulatory region comprising between 1 nucleotide and 10 kB of sequence extending in a 5' direction from the start codon of the gene.

26. The transgenic nematode of claim 1, wherein the detectable marker is expressed in a dorsal pharyngeal gland.

27. The transgenic nematode of claim 1, wherein the gene is expressed in a dorsal pharyngeal gland.

28. The transgenic nematode of claim 1, wherein the detectable marker is expressed in the subventral pharyngeal gland.

5 29. The transgenic nematode of claim 1, wherein the gene is expressed in the subventral pharyngeal gland.

30. The transgenic nematode of claim 1, wherein the detectable marker is expressed in an amphid sheath cell.

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31. The transgenic nematode of claim 1, wherein the gene is expressed in an amphid sheath cell.

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32. The transgenic nematode of claim 1, wherein the gene encodes a member of the venom allergen protein family.

33. The transgenic nematode of claim 1, wherein the gene is *C. elegans vap-1*.

34. The transgenic nematode of claim 1, wherein the gene is *C. elegans vap-2*.

35. The transgenic nematode of claim 1, wherein the gene is a member of the *C. elegans vap* family.

36. A method of identifying a compound that inhibits a nematode secretion pathway comprising steps of:

providing a nematode, wherein the nematode produces a secretion product;

contacting the nematode with a test compound; and

detecting a decrease in the activity or amount of the secretion product when the test compound is present versus when the test compound is absent.

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37. A method of identifying a compound that inhibits a nematode secretion pathway comprising steps of:

providing a population of nematodes, wherein the nematodes produce a secretion product;

5 dispensing approximately equal numbers of the nematodes into a plurality of vessels;

contacting the nematodes in each of one or more of the vessels with one or more test compounds; and

10 detecting a decrease in the activity or amount of the secretion product in one or more of the vessels when the one or more test compounds are present versus when the one or more test compounds are absent.

15 38. The method of claim 37, wherein the vessels are wells of one or more multiwell plates.

39. The method of claim 36, wherein the nematode secretion product is a polypeptide.

40. The method of claim 36, wherein the detecting step comprises detecting the secretion product in a culture medium in which the nematode is cultured.

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41. The method of claim 36, wherein the nematode is *C. elegans*.

42. The method of claim 36, wherein the providing step comprises providing the nematode of claim 1.

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43. The method of claim 42, wherein the detecting step comprises detecting fluorescence.

44. The method of claim 43, wherein the transgene encodes a reporter molecule capable of generating light emission that is detectable above background light generated by host cell molecules other than the reporter molecule.

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45. The method of claim 43, wherein the transgene encodes a reporter molecule that fluoresces under predetermined conditions, and wherein the fluorescence is detectable above background fluorescence emitted by host cell molecules other than the reporter molecule.

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46. A method of generating a nematode comprising steps of:

- (a) selecting a parasitic nematode secretory protein;
- (b) identifying a *C. elegans* homolog of the protein selected in step (a);
- (c) identifying a nucleic acid sequence comprising a regulatory region of a *C.*

elegans gene encoding the *C. elegans* homolog identified in step (b); and

(d) generating a transgenic nematode, wherein cells of the transgenic nematode comprise a nucleic acid sequence including the identified regulatory region operably linked to a nucleic acid sequence encoding a detectable marker .

47. The method of claim 46, wherein the parasitic nematode is a *Tylenchid* nematode.

48. The method of claim 46, wherein the regulatory region comprises a promoter of the *C. elegans* homolog identified in step (b).

49. The method of claim 46, wherein the nucleic acid sequence of step (d) includes at least a portion of the coding sequence of a gene encoding the *C. elegans* homolog of part (c).

50. The method of claim 49, wherein the nucleic acid sequence of step (d) includes a signal sequence.

51. The method of claim 49, wherein the nucleic acid sequence of step (d) includes at least a portion of an intron from a gene encoding the *C. elegans* homolog of part (c).

52. The method of claim 49, wherein the nucleic acid sequence of step (d) includes at least a portion of the 3' untranslated region from a gene encoding the *C. elegans* homolog of part (c).

5 53. The method of claim 46, wherein the regulatory region is sufficient to direct expression of the nucleic acid of step (d).

54. The method of claim 53, wherein the regulatory region directs expression of the nucleic acid of step (d) so that the nucleic acid is expressed in a pharyngeal gland cell or an amphid sheath cell.

55. A pharmaceutical composition comprising:

a compound identified according to the method of claim 36; and
a pharmaceutically acceptable carrier.

56. An anti-nematode agent for use in preventing or reducing nematode infestation of a plant comprising:

a compound identified according to the method of claim 36; and
an agriculturally acceptable carrier.

57. A method of treating or reducing the likelihood of nematode infection in an individual comprising the steps of:

identifying an individual at risk of or suffering from a nematode infection; and
administering the pharmaceutical composition of claim 55.

58. A method of preventing or reducing nematode infestation of a plant comprising the steps of:

identifying a plant at risk of nematode infestation; and
applying the anti-nematode agent of claim 56 to the plant or to the vicinity of the

plant.

59. A method of preventing or reducing nematode infestation of a plant comprising the step of:

treating soil in which a plant is to be grown with the anti-nematode agent of claim

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60. A method of preventing or reducing nematode infestation of a plant comprising the step of:

treating a seed from which a plant is to be grown with the anti-nematode agent of claim 56.

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61. A method of identifying a target for anti-nematode compound development comprising the steps of:

providing an assay for a nematode secretion pathway;

mutagenizing a population of nematodes; and

15 identifying, using the assay, a mutant nematode with an alteration in the nematode secretion pathway, wherein the nematode has a mutation in a gene.

62. The method of claim 61, further comprising the step of:

20 identifying the gene, thereby identifying a target for anti-nematode compound development.

63. The method of claim 62, further comprising the step of:

cloning the gene, thereby identifying a target for anti-nematode compound development.

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64. The method of claim 62, wherein the target is the identified gene.

65. The method of claim 62, wherein the target is a polypeptide encoded by the gene.

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66. The method of claim 61, wherein the population of nematodes comprises *C. elegans* nematodes.

67. The method of claim 61, wherein the population of nematodes comprises the transgenic nematode of claim 1.

5 68. The method of claim 61, wherein the assay comprises detecting a nematode secretion product.

69. The method of claim 61, wherein the nematode secretion product includes a detectable marker.

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70. The method of claim 68, wherein the assay comprises detecting a nematode secretion product in medium in which the nematode is cultured.

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71. The method of claim 68, wherein the assay comprises detecting a nematode secretion product within the nematode.

72. A mutant nematode identified according to the method of claim 61.

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73. A vector comprising a polynucleotide that encodes a polypeptide, the amino acid sequence of which comprises the sequence of SEQ ID NO:1.

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74. The vector of claim 73, further comprising a regulatory element comprising a polynucleotide sequence comprising the *C. elegans vap-1* promoter operably linked to the polynucleotide that encodes a polypeptide, the amino acid sequence of which comprises the sequence of SEQ ID NO:1.

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75. The vector of claim 74, wherein the regulatory element is a 5' regulatory region comprising between 1 nucleotide and 10 kB of sequence extending in a 5' direction from the start codon of the *C.elegans vap-1* gene.

76. The vector of claim 73, further comprising a polynucleotide sequence that encodes a detectable marker in frame with the polynucleotide.

77. A vector comprising a polynucleotide that encodes a polypeptide, the amino acid sequence of which comprises at least 10 consecutive residues of SEQ ID NO:1.

78. A vector comprising a polynucleotide that encodes a polypeptide, the amino acid sequence of which comprises a sequence at least 50% identical to SEQ ID NO:1.

79. A vector comprising a regulatory element comprising a polynucleotide sequence comprising the *C. elegans vap-1* promoter operably linked to a polynucleotide encoding a detectable marker.

80. The vector of claim 79, wherein the regulatory element comprises a regulatory region comprising between 1 nucleotide and 10 kB of sequence extending in a 5' direction from the start codon of the *C.elegans vap-1* gene.

81. The vector of claim 79, further comprising at least a portion of the *C. elegans vap-1* gene, wherein the regulatory element is operably linked to the portion.

82. A vector comprising the polynucleotide sequence of SEQ ID NO:2.

83. A vector comprising a polynucleotide that encodes a polypeptide, the amino acid sequence of which comprises the sequence of SEQ ID NO:3.

84. The vector of claim 83, further comprising a regulatory element comprising a polynucleotide sequence comprising the *C. elegans vap-1* promoter operably linked to the polynucleotide that encodes a polypeptide, the amino acid sequence of which comprises the sequence of SEQ ID NO:3.

85. The vector of claim 84, wherein the regulatory element is a 5' regulatory region comprising between 1 nucleotide and 10 kB of sequence extending in a 5' direction from the start codon of the *C.elegans vap-1* gene.

5 86. The vector of claim 83, further comprising a polynucleotide sequence that encodes a detectable marker in frame with the polynucleotide sequence of claim 500.

87. A vector comprising a polynucleotide that encodes a polypeptide, the amino acid sequence of which comprises at least 10 consecutive residues of SEQ ID NO:3.

10 88. A vector comprising a polynucleotide that encodes a polypeptide, the amino acid sequence of which comprises a sequence at least 50% identical to SEQ ID NO:3.

15 89. A vector comprising a regulatory element comprising a polynucleotide sequence comprising the *C. elegans vap-2* promoter operably linked to a polynucleotide encoding a detectable marker.

20 90. The vector of claim 89, wherein the regulatory element comprises a regulatory region comprising between 1 nucleotide and 10 kB of sequence extending in a 5' direction from the start codon of the *C.elegans vap-2* gene.

91. The vector of claim 89, further comprising at least a portion of the *C. elegans vap-2* gene, wherein the regulatory element is operably linked to the portion.

25 92. A vector comprising the polynucleotide sequence of SEQ ID NO:4.

93. A vector comprising a regulatory element comprising a polynucleotide sequence comprising the promoter of a gene belonging to the *C. elegans vap* family of genes operably linked to a polynucleotide encoding a detectable marker.

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94. The vector of claim 93, wherein the regulatory element comprises a regulatory region comprising between 1 nucleotide and 10 kB of sequence extending in a 5' direction from the start codon of a gene belonging to the *C.elegans vap* family of genes.

5 95. The vector of claim 94, further comprising at least a portion of the coding region of the gene, wherein the regulatory region is operably linked to the portion.

96. A method of expressing a first polynucleotide in a *C. elegans* amphid sheath cell comprising the step of:

10 introducing into the *C. elegans* amphid sheath cell a first polynucleotide comprising a *vap-1* regulatory region operably linked to the second polynucleotide.

97. A method of expressing a polypeptide in a *C. elegans* amphid sheath cell comprising the step of:

15 introducing into the *C. elegans* amphid sheath cell a first polynucleotide comprising a *vap-1* regulatory region operably linked to a second polynucleotide that encodes the polypeptide.

20 98. The method of claim 96, wherein the *vap-1* regulatory region comprises between 1 nucleotide and 10kB of sequence extending in a 5' direction from the start codon of the *C. elegans vap-1* gene.

25 99. The method of claim 96, wherein the introducing step comprises injecting a polynucleotide into a *C. elegans* worm, wherein the polynucleotide comprises the first polynucleotide operably linked to the second polynucleotide .

100. The method of claim 96, wherein the introducing step comprises generating a transgenic nematode the cells of which comprise the first polynucleotide operably linked to the second polynucleotide.

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101. A method of expressing a first polynucleotide in a *C. elegans* amphid sheath cell comprising the step of:

introducing into the *C. elegans* amphid sheath cell a first polynucleotide comprising a *vap-2* regulatory region operably linked to the second polynucleotide.

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102. A method of expressing a polypeptide in a *C. elegans* amphid sheath cell comprising the step of:

introducing into the *C. elegans* amphid sheath cell a first polynucleotide comprising a *vap-2* regulatory region operably linked to a second polynucleotide that encodes the polypeptide.

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103. The method of claim 101, wherein the *vap-2* regulatory region comprises between 1 nucleotide and 10kB of sequence extending in a 5' direction from the start codon of the *C. elegans vap-2* gene.

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104. The method of claim 101, wherein the introducing step comprises injecting a polynucleotide into a *C. elegans* worm, wherein the polynucleotide comprises the first polynucleotide operably linked to the second polynucleotide .

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105. The method of claim 101, wherein the introducing step comprises generating a transgenic nematode the cells of which comprise the first polynucleotide operably linked to the second polynucleotide.